Intraluminal EWSR1-CREB1 gene rearranged, low-grade myxoid sarcoma of the pulmonary artery resembling extraskeletal myxoid chondrosarcoma (EMC)

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Intraluminal *EWSR1-CREB1* gene rearranged, low-grade myxoid sarcoma of the pulmonary artery resembling extraskeletal myxoid chondrosarcoma (EMC)

Running Title: *EWSR1-CREB1* sarcoma of the pulmonary artery

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Fusions of the *EWSR1* gene with partners from the *CREB* gene family have been identified in several histologic entities, mostly mesenchymal tumors of variable clinical malignant potential[1], including angiomatoid fibrous histiocytomas (AFH), clear cell sarcomas (CCS), clear cell sarcoma-like tumors of the gastrointestinal tract (CCSLTGT), primary pulmonary myxoid sarcoma (PPMS)[2], intracranial myxoid neoplasias (iMN)[3, 4] and rare carcinoma variants. Interestingly, some of *EWSR1-CREB*-family gene fusion associated tumors show a prominent myxoid component and some of such tumors were histologically in the past

diagnosed as "consistent with extraskeletal myxoid chondrosarcomas (EMC)" [3, 5]. EMC is a rare sarcoma, currently defined as a myxoid mesenchymal tumor carrying the translocation of *NR4A3* gene with one of several partner genes[6], *EWSR1* gene being one of the most common. Since *EWSR1* gene is involved in the pathogenesis of aggressive Ewing sarcoma, *EWSR1* FISH has been broadly available for some time for subtyping sarcomas. In the past, FISH evidence of the *EWSR1* rearrangement in a myxoid mesenchymal neoplasm with compatible microscopic properties was often interpreted as being diagnostic for EMC. More recent findings, however, have proven, that some of these tumors do not belong to the *EWSR1-NR4A3* category, but rather to the *EWSR1-CREB* family translocated tumor category, thus mandating caution in the interpretation of *EWSR1* FISH in myxoid tumors. At present, the exact topographic distribution of myxoid mesenchymal *EWSR1-CREB* translocated tumors is not well known, due to the rarity of the entity as well as, the inherent diagnostic challenges. Recent reports have described such tumors as being mostly intrapulmonary (PPMS) [2, 5] or intracranial (iMN) [3, 4, 7] with one perirectal example [7]. Hybrid tumors, with a typical AFH areas and a variable myxoid component have been described [4, 8, 9] in a wide range of locations.

Intraluminal tumors of large vessels or the heart are rare. They correspond predominantly to undifferentiated pleomorphic sarcomas, designated as intimal sarcomas, showing amplification of the *MDM2* gene[10]. There are rare reports on myxoid tumors in the large vessels or in the heart, mostly corresponding to pleomorphic sarcomas with focal myxoid change [11]. However, some of such tumors are referred to as of low-grade malignancy [12, 13]. Molecular characterization in these reports is lacking. There is only one report on an intraluminal tumor of pulmonary artery morphologically corresponding to AFH, which was found to contain *EWSR1-ATF1* gene fusion [14]. In the current report, we describe a well-characterized, low grade myxoid, exclusively intraluminal tumor of the pulmonary artery with an *EWSR1-CREB1* gene fusion and a long follow up.

Histopathological material was retrieved from the pathology files of the Institute of Pathology, University Hospital, Zurich, Switzerland. Clinical and follow-up data were obtained from clinical databases of the Department of Thoracic Surgery. The study was approved by our institutional review board (the Cantonal Ethics Committee; KEK_ZH 2013_0430). Tumor tissue samples were fixed in buffered 4% formalin and embedded in paraffin. Immunohistochemistry (IHC) using the broad spectrum cytokeratin (AE1/AE3, Dako, Baar, Switzerland), SMA (1A4, Sigma, Darmstadt, Germany) and Mib1 (30-9, Ventana Roche, Basel, Switzerland) antibodies was performed using the Ventana Benchmark XT automated system (Ventana Medical Systems, Tucson, Arizona). Fluorescence In Situ Hybridization (FISH) studies were performed with dual color break-apart FISH detecting translocations of the *EWSR1* (Vysis, Abbott AG, Baar, Switzerland) and *NR4A3* (ZytoVision GmbH, Bremerhaven, Germany) genes as wells as dual color FISH detecting copy numbers of the centromeric region of the chromosome 12 and of the *MDM2* gene for the evaluation of the amplification of the *MDM2* gene (ZytoVision GmbH, Bremerhaven, Germany) were performed. Next Generation Sequencing (NGS) of the tumor samples was performed as previously described with the commercially available Archer FusionPlex Sarcoma Panel (ArcherDx, Boulder, CO, USA), which simultaneously identifies fusions of 26 genes associated with soft tissue neoplasias [15].

Clinical history: A 21-year old female presented with unspecific symptoms and deterioration in physical performance since few weeks. Laboratory work up disclosed elevated inflammatory parameters, thrombocytosis and anemia. Pulmonary function test showed significant restriction. Physical exam revealed abnormal systolic heart murmurs. Echocardiography showed pericardial fluid and a large mass in the pulmonary artery (PA). CT with angiography, MRI and ventilation-perfusion scan demonstrated a mass in the main trunk of the PA with slight metabolic uptake in the PET/CT scan **(Figure 1 A)**. Endobronchial ultrasound-guided fine-needle aspiration contained abundant myxoid tumor tissue with low

cellularity and the lack of necrosis or mitotic activity. Immunohistochemically, unspecific immunoprofile was seen, with weak expression of cytokeratin (AE1/AE3) **(Figure 2 A)** and no nuclear expression of MDM2 protein as well as a low proliferation rate **(Figure 2 C)**. Cytopathological findings were diagnostic of a myxoid mesenchymal neoplasia, likely of low-grade malignant potential. The findings were not diagnostic of an intimal sarcoma, as the features of a high-grade sarcoma were lacking. Following an interdisciplinary discussion, the decision was made to completely resect the tumor in curative intention, as there were no signs of metastatic disease. Pulmonary tumor endarterectomy **(Figure 1 B, C)** with reconstruction of the right PA in deep hypothermia was performed. The postoperative course was uneventful and the patient was discharged on the 6th postoperative day. No adjuvant therapy was administered. The last regular follow up visit took place at 38 months after the first diagnosis, without clinical or imaging evidence of recurrence or metastatic disease. The patient has returned to her usual preoperative level of physical activity receiving lifelong anticoagulation. Several fragments of friable, myxoid, beige tumor tissue were submitted for histopathological analysis. Microscopically **(Figure 2 A-C)**, abundant hypovascular, myxoid matrix was seen with focal hemosiderin deposition and a heterogeneous proliferation of middle-sized tumor cells, forming trabecular networks as well as rare solid areas. There was no lobulation, septation, inflammatory infiltration or necrosis. The tumor cells contained oval nuclei and a variable amount of eosinophilic cytoplasm, mostly with discernable cell borders. In selected solid areas, a more pronounced nuclear atypia was seen, which however was not accompanied by increased mitotic activity **(Figure 2 C)**. Immunohistochemistry showed focal cytokeratin (AE1/AE3) **(Figure 2 A),** synaptophysin and SMA **(Figure 2 B)** expression, while reactions for EMA, S100, desmin, ERG, MDM2 and CDK4 were negative. Nuclear expression of INI1 protein was retained. The MiB1 proliferation index was low (<10%). *EWSR1* and *MDM2* gene FISH analyses were performed at initial diagnosis and showed rearrangement of the *EWSR1* gene **(Figure 2 D)**, without amplification of the *MDM2* gene **(Figure 2 F)**. Rearrangement of the *EWSR1* gene was considered compatible with the morphological diagnosis of EMC. Since molecular testing of the *NR4A3* gene rearrangement

was not available at initial diagnosis, the molecular background of the tumor could not be further clarified. Upon availability, *NR4A3* FISH and NGS were performed retrospectively on the resected tumor specimen. No rearrangement of the *NR4A3* gene **(Figure 2 E)** was found on FISH; however, NGS analysis demonstrated a *EWSR1-CREB1* fusion as the sole rearrangement.

Tumors of the pulmonary artery often show prominent or even predominant intraluminal growth in the form of clinically aggressive intimal sarcomas, that are often associated with amplifications of the 12q13-15 chromosomal region[10]. Low-grade malignant tumors are less common in these locations [12, 13] and there are few data on their genetic background. In fact, there is only one report of a purely intraluminal primary tumor of a pulmonary artery in a 76 year-old woman, showing microscopical features of AFH and carrying the *EWSR1-ATF1* gene fusion[14]. In the current study, we report on a purely myxoid, low-grade tumor with *EWSR1-CREB1* fusion, confined to the lumen of the pulmonary artery in a 21-year old woman. Both *ATF1* and *CREB1* genes belong to the CREB family of transcription factors[1]. Fusions of *EWSR1* with one of the genes of the *CREB* family occur in clinically heterogeneous, mostly mesenchymal neoplasia, comprising AFHs and other rare myxoid tumors, currently designated according to site of occurrence: tumors in the lung are referred to as "primary pulmonary myxoid sarcomas" (PPMS) [2, 5] and morphologically identical intracranial tumors as "intracranial myxoid neoplasm" (iMN) [3, 7, 16]. Only one such purely myxoid tumor outside of these two locations was reported in the perirectal region [7]. Even if non-specific, the immunoprofile of the current tumor does not fit well into the PPMS or myxoid AFH diagnostic categories, as the latter lack the expression of cytokeratins. Several earlier studies reported hybrid tumors containing gene fusions between *EWSR1* and one of the genes of the *CREB*-family, so called "myxoid AFH" [8, 9] , described both within the lung and intracranial locations [4, 17, 18]. Clinical data suggest that all three categories of the *EWRS1-CREB* family genes fusion carrying tumor types (pure AFH, pure myxoid and hybrid

AFH-myxoid variants) show low-grade malignant potential. Of the two pulmonary artery tumor with *EWSR1-CREB* family gene fusions, both were pure forms: an *EWSR1-ATF1* AFH described by Ghinga[14] and an exclusively myxoid tumor with *EWSR1-CREB1* fusion from the current study. During the 38 months of follow up, there has been no evidence of local recurrences or metastases in our patient, while follow up data are not provided for the patient with the previously described AFH. Since there are no descriptions of hybrid forms in this location, it remains unclear, weather these two tumors are in fact within the spectrum of the same entity.

Initial histopathological evaluation of the present case pointed out towards the diagnosis of an EMC. In view of the increased availability of molecular tools for routine diagnostics in recent years, reevaluation of the diagnoses of soft tissue and bone tumors is often warranted[19]. Currently, histopathologic diagnosis of EMC is based on the demonstration of a rearrangement of the *NR4A3*[6]. This genetic aberration has only become recently available for routine diagnostics, so that the EMC diagnosis was considered one of exclusion, in some cases supported by the demonstration of the rearrangement of the *EWSR1* gene (as in our case). In our case, extended FISH and NGS analyses demonstrated the lack of the rearrangement of the *NR4A3* gene and the *CREB1* gene was identified as the fusion partner of the rearranged *EWSR1* gene, removing the tumor from the category of the EMC. Morphologically comparable tumor, diagnosed as an EMC was described in 2010[20] in the heart of a 26-year-old man, however the tumor disease was widely metastatic, so contrary to our case, it remains unresolved if this is a primary or metastatic heart tumor. The *EWSR1* gene FISH in this case was negative and no further molecular studies were done. Yet another tumor reported as an EMC of PA[21] is obviously incorrectly diagnosed as such, since no molecular studies were performed and the microphotographs illustrate hyaline chondrogenic neoplasia, immunohistochemically strongly expressing S100 protein, findings excluding the diagnosis of EMC.

In conclusion we describe a well-documented tumor with an *EWSR1-CREB1* fusion, which has never previously been reported in a purely intraluminal tumor of large vessels or heart. This report widens the spectrum of possible distributions of *EWSR1-CREB*-family rearranged myxoid tumors on one hand, and the spectrum of differential diagnoses of primary intravascular neoplasias of large vessels on the other.

Legends

Figure 1: (A) PET-CT showed slight metabolic activity of the intraluminal pulmonary artery mass (arrow) **(B, C)** Intraoperativelly, a gelatinous tumor (asterisk), freely floating in the vessel and attached to the left pulmonary artery wall was found and completely resected.

Figure 2: (A) Intraluminal tumor mass of the pulmonary artery consisted of polypoid tumor tissue with predominantly low cellularity (H&E; 50x) and focal cytokeratin AE1/AE3 expression (inset; 400x). **(B)** Middle sized spindle cells were embedded in prominent myxoid extracellular matrix and showed a partial trabecular growth pattern (H&E; 400x) with focal SMA expression (inset; 400x). **(C)** Focal increased cellularity with mild atypia could be observed, however the proliferation rate was low (inset, Mib1; 400x). **(D)** Break apart FISH of the *EWSR1* gene showed one fused signal (arrowhead) and one split pair of red and green signals (arrows) in the nuclei of the tumor cells, indicating rearrangement of the *EWSR1* gene. **(E)** Tumor cell nuclei in the break apart FISH analysis of the *NR4A3* gene contained exclusively fused signals (arrowheads), suggesting absence of the rearrangement of the *NR4A3* gene. **(F)** No amplification of the *MDM2* gene was observed in the FISH study.

References:

1. Thway K, Fisher C. Tumors with ewsr1-creb1 and ewsr1-atf1 fusions: The current status. *Am J Surg Pathol* 2012;**36**;e1-e11.
2. Thway K, Nicholson AG, Lawson K *et al.* Primary pulmonary myxoid sarcoma with ewsr1-creb1 fusion: A new tumor entity. *Am J Surg Pathol* 2011;**35**;1722-1732.
3. Velz J, Agaimy A, Frontzek K *et al.* Molecular and clinicopathologic heterogeneity of intracranial tumors mimicking extraskeletal myxoid chondrosarcoma. *J Neuropathol Exp Neurol* 2018;**77**;727-735.
4. Bale TA, Oviedo A, Kozakewich H *et al.* Intracranial myxoid mesenchymal tumors with ewsr1-creb family gene fusions: Myxoid variant of angiomatoid fibrous histiocytoma or novel entity? *Brain Pathol* 2018;**28**;183-191.
5. Matsukuma S, Hisaoka M, Obara K *et al.* Primary pulmonary myxoid sarcoma with ewsr1-creb1 fusion, resembling extraskeletal myxoid chondrosarcoma: Case report with a review of literature. *Pathology International* 2012;**62**;817-822.
6. Lucas DR, Stenman G. Extraskeletal myxoid chondrosarcoma. In Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F eds. *Who classification of tumours of soft tissue and bone*. Lyon: IARC, 2013;223-224.
7. Kao YC, Sung YS, Zhang L *et al.* Ewsr1 fusions with creb family transcription factors define a novel myxoid mesenchymal tumor with predilection for intracranial location. *Am J Surg Pathol* 2017;**41**;482-490.
8. Schaefer IM, Fletcher CD. Myxoid variant of so-called angiomatoid "malignant fibrous histiocytoma": Clinicopathologic characterization in a series of 21 cases. *Am J Surg Pathol* 2014;**38**;816-823.
9. Chen G, Folpe AL, Colby TV *et al.* Angiomatoid fibrous histiocytoma: Unusual sites and unusual morphology. *Modern Pathology* 2011;**24**;1560.
10. Bode-Lesniewska B, Debies-Rychter M. Intimal sarcoma. In Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F eds. *Who classification of tumours of soft tissue and bone*. Lyon: IARC, 2013;232-233.
11. Burke A, Tavora FR, Maleszewski JJ, Frazier AA. *Tumors of the heart and great vessels*. Silver Spring, Maryland: American Registry of Pathology, 2015.
12. Suh JH, Kim DY, Yoon JS, Park ES, Park CB. Low grade myxofibrosarcoma in the right ventricle presenting as pulmonary thromboembolism. *J Thorac Dis* 2017;**9**;E1084-E1087.
13. Gupta P, Jain M. Right-sided low grade myxofibrosarcoma of the heart in an adult. *Asian Cardiovascular and Thoracic Annals* 2013;**21**;208-210.
14. Ghigna M-R, Hamdi S, Petitpretz P *et al.* Angiomatoid fibrous histiocytoma of the pulmonary artery: A multidisciplinary discussion. *Histopathology* 2014;**65**;278-282.
15. Velz J, Agaimy A, Frontzek K *et al.* Molecular and clinicopathologic heterogeneity of intracranial tumors mimicking extraskeletal myxoid chondrosarcoma. *Journal of Neuropathology & Experimental Neurology* 2018;**77**;727-735.
16. Sciot R, Jacobs S, Calenbergh FV, Demaerel P, Wozniak A, Debiec-Rychter M. Primary myxoid mesenchymal tumour with intracranial location: Report of a case with a ewsr1–atf1 fusion. *Histopathology* 2018;**72**;880-883.
17. Gareton A, Pierron G, Mokhtari K *et al.* Eswr1-crem fusion in an intracranial myxoid angiomatoid fibrous histiocytoma-like tumor: A case report and literature review. *Journal of Neuropathology & Experimental Neurology* 2018;**77**;537-541.
18. Smith SC, Palanisamy N, Betz BL *et al.* At the intersection of primary pulmonary myxoid sarcoma and pulmonary angiomatoid fibrous histiocytoma: Observations from three new cases. *Histopathology* 2013;**65**;144-146.
19. Noujaim J, Jones RL, Swansbury J *et al.* The spectrum of ewsr1-rearranged neoplasms at a tertiary sarcoma centre; assessing 772 tumour specimens and the value of current ancillary molecular diagnostic modalities. *Br J Cancer* 2017;**116**;669-678.

20. Geyer HL, Karlin N. Extraskeletal myxoid chondrosarcoma of the heart and review of current literature. *Curr Oncol* 2010;**17**;58-62.
21. Gadabanahalli K, Belaval VV, Bhat V, Gorur IM. Primary extraskeletal myxoid chondrosarcoma of pulmonary arteries: A rare mimic of acute pulmonary thromboembolism. *Interact Cardiovasc Thorac Surg* 2015;**20**;565-566.